Laboratory of Environmental Mutagenesis:
Summary Statement

by H. V. Malling

The Laboratory of Environmental Mutagenesis (LEM) is one of the three scientific laboratories at the National Institute of Environmental Health Sciences.

In the formation of the programs for such a laboratory, it is important to set some goals and then start to ask the "how and why" questions.

The societal goal for the LEM is the prevention of increased frequency of genetic disease in the human population. That goal covers the waterfront; however, the LEM cannot possibly cover all the scientific areas necessary to accomplish that goal. Independently of how large we will grow, there will be areas left out.

The next questions then become, "Which parts are we going to do and how are we going to do them?" Our approaches include: (1) development of short-term testing systems for mutagenic activity, (2) development of mammalian mutation systems which can be used to predict the risk for the human population, (3) development of monitoring systems for detection of mutations in the human population, and (4) evaluation of the fitness of populations with an increased number of mutations in the gene pool.

With regard to development of short-term tests for mutagenesis screening, some pollutants in the environment are neither mutagenic nor carcinogenic by themselves but can be converted by mammalian metabolism to highly reactive and genetically active metabolites. Microorganisms have only a fraction of the toxification-detoxification mechanisms that a mammal has. To screen for mutagenicity in mammals, however, is extremely expensive and time-consuming, whereas many quantitative mutation systems exist today in microorganisms. Therefore, in the development of the short-term tests we have to combine the microbial mutation system with the drug metabolism of the higher animal. This can be done by homogenizing organs of laboratory animals and preparing various enzyme fractions and testing them for their ability to form active metabolites from non-active parent compounds. But by homogenizing an organ and fractionating it into various parts, the efficiency of the activation pathways and balance between the toxification and detoxification mechanisms may be destroyed. This problem can be avoided by injecting the indicator organism into the bloodstream of the animal and treating the animal with the compound under test and later isolating the microorganism and measuring mutations in them.

The microorganisms, however, are not inert components, lacking any metabolism. Since they do have their own activation and detoxification systems, it is very important to know which part of the activation is due to the microorganism and which part is due to mammalian metabolism. This problem is being studied in the LEM in collaboration with the Laboratory of Pharmacology.

Because microorganisms have a cell wall which can prevent penetration of the cells by certain chemicals, some chemicals which are active in mammalian cells are inactive in microorganisms. To circumvent this problem in the microbial tests, we are attempting to isolate yeast cells which are more permeable.

Microbial systems alone obviously cannot be used to predict the genetic hazard to the human population from exposure to environmental agents. Mammalian systems are also required.

One of the very important mutation systems in mammals is the measurement of induced transmissible translocations. In the government document on mutagenesis it is stated that if a compound increases the frequency of translocation in mammals, it is to be considered as a human mutagen. This is the type of chromosomal mutations in the human population that may result in various types of mental retardation. Presently we are studying the effect of the unbalanced genome in the offspring of a

translocation heterozygote on the development of the embryo. Hopefully, these studies will lead to a much deeper understanding of the hazard to the health of offspring of the individuals carrying various types of translocations.

A new mammalian mutation system has been developed; namely, the biochemical specific locus mutation system in which mutations can be detected in specific enzymes. At present, we are only working with nine different genes, but have a breeding program with The Jackson Laboratory to increase this number to 50. At present we are studying the model, triethylenemelamine, in this system and have isolated many new mutants.

In contrast to other specific locus mutation systems in mammals, the biochemical specific locus mutation system has a close parallel in man; because humans and mice have many of the same enzymes. This system will most likely add to our ability to make predictions about the genetic hazard of a certain compound to the human population from our animal data.

In order to do this with even more reliability, we have just started a unit for sequencing proteins and later RNA and DNA. This unit will also sequence the mutant proteins to identify the genetic alterations in mutants. The different types of genetic alterations could have quite different effects on the individuals which carry them. Dr. Neel, in Ann Arbor, Michigan, is trying to monitor the human population for mutations by enzyme analysis. Many of the enzymes in which we are measuring mutations overlap with Dr. Neel's battery of enzymes. To a certain degree we are providing the background data which will help to evaluate the findings in the human population.

Knowingly or unknowingly, fractions of the human population are exposed to hazardous compounds. It is important, therefore, to have a system for detection of mutations in the human population. Let us first consider Neel's system in which the offspring are screened in order to determine the mutant frequency of the parents. This system requires babies from perhaps a million people to come up with any statistical significance on a reasonable increase in mutation frequency. This system can give a general trend in the population; and while that is important, the epidemiological unit we are working with is too gross to pinpoint specific hazardous environments smaller in area than New York. This problem occurs because the individual is just one event. If instead we could estimate the mutation frequency on an individual we would be in much better shape. To do this we are developing systems to measure mutations in single cells *in vivo*. Here we are limited too; the only two types of samples of single cells we can get easily from humans are cells in blood and semen.

In collaboration with Dr. Stamatoyannopoulos at the University of Washington, Seattle, we have developed a technique for detecting RBC's which carry the sickle cell hemoglobin. The technique is based on development of monospecific antibodies to S hemoglobin and labeling it with a fluorescent molecule. The only cell which will react with this antibody is the sickle cell. In any normal individual there are approximately 10^{-7} sickle cells. This number could be expected to go up by exposure to mutagens. The mutation which results in S hemoglobin is only one type of many hemoglobin variants. Dr. Stamatoyannopoulos, therefore, is making antibodies to many other hemoglobin mutants. This will increase the sensitivity of the system. In order to interpret the data we need animal model systems. Various strains of mice contain different hemoglobins and we are utilizing these differences to develop such model systems.

Around the midpiece of sperm is a sheet of mitochondria containing enzymes which can be detected by histochemical stains. By using specific inhibitors of these enzymes, it may be possible to detect mutation resistance to the inhibitor. I hope to use this mutation detecting technique to develop a monitoring system for the human population.

One of the advantages of the RBC system and the sperm system is that both are nondestructive to the animal and the detection of mutations, therefore, can piggyback on any bigger chronic feeding study. In this way the mutation frequencies also can be correlated with other toxicological phenomena such as tumorgenesis, etc. When this correlation is known, the monitoring system based on detection of mutants in the RBC's could also serve as an early warning system for certain cancers in the human population.

There are many harmful chemicals that we cannot avoid in the environment; some of these may be mutagenic. The human society must set limits to chemical exposure as was done for irradiation. In order to evaluate the health hazard imposed on the human population by an increase in mutation frequency, we must evaluate the fitness of individuals carrying these mutations in their genome. Drosophila is the only available organism in which this can be done. In the Population Genetics Section we are comparing the fitness of the mutations which exist in the natural population and, therefore, has been exposed to some selection with the fitness of newly induced mutations. The data which will come out of these comparisons should help us evaluate the genetic hazard imposed on the human population by exposure to mutagens.